

Biofilm system 3

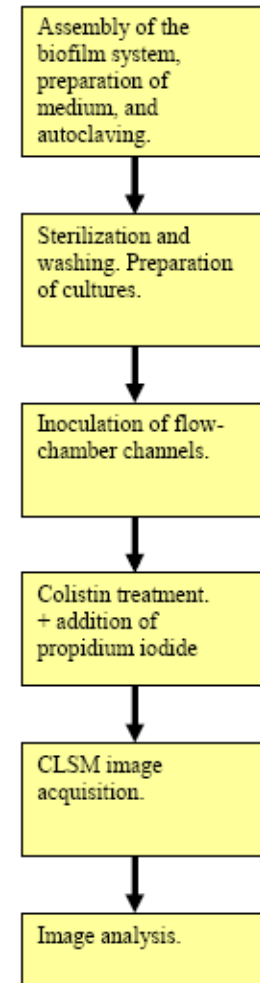
Group 1

Purpose of the biofilm system 3

- To study differentiation and colistin tolerance in *Pseudomonas aeruginosa* biofilms
- Background:
 - Glucose-grown *P. aeruginosa* forms mushroom shaped biofilms due to motile and non-motile subpopulations (Klausen *et al.* 2003)
 - Biofilms possess high tolerance to antimicrobial agents such as colistin and it is shown to be due to motile subpopulations in mushroom shaped *P. aeruginosa* biofilms (Haagensen *et al.* 2007)

Experiment briefly

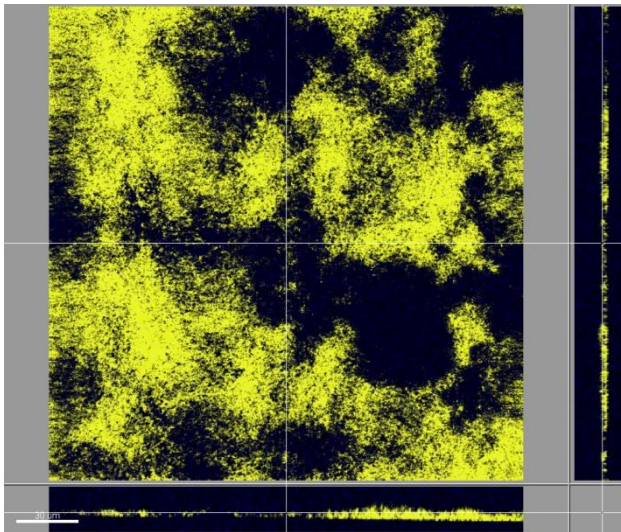
- Flow channels:
 - Untreated *P. aeruginosa* (Gfp)
 - Colistin treated *P. aeruginosa* (Gfp)
 - Mixture of *P. aeruginosa* (Yfp) and *P. aeruginosa* pilA (Cfp)



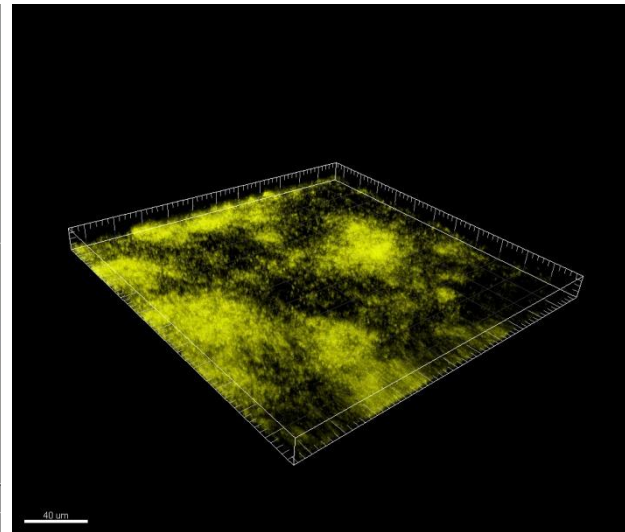
Expected results

- Mushroom shaped biofilms in mixed biofilms
 - non-motile (pilA) and motile (wt) subpopulations forms stalk and cap of the biofilm mushrooms, respectively
- Increased tolerance to colistin

Differentiation in mixed biofilms

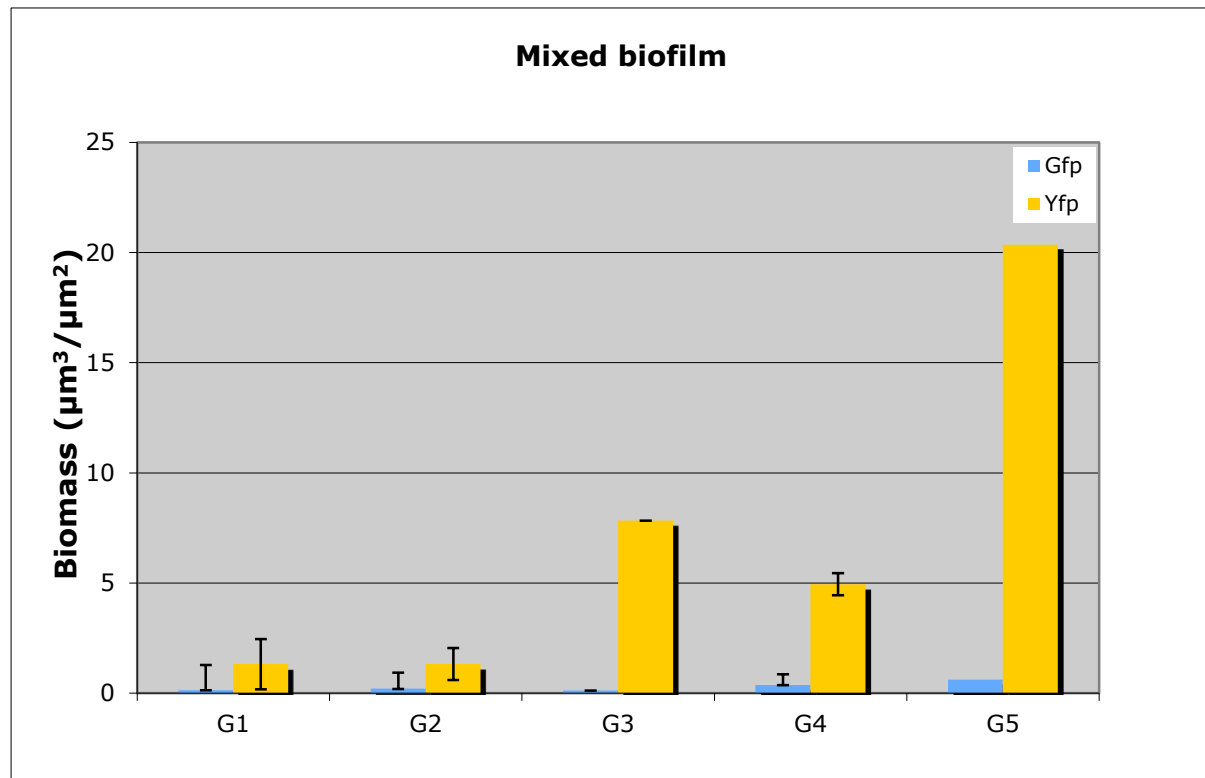


Group 5



Group 2

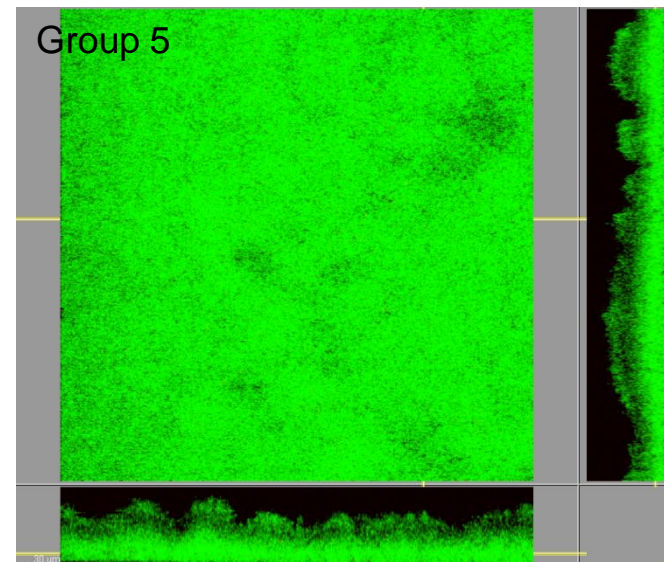
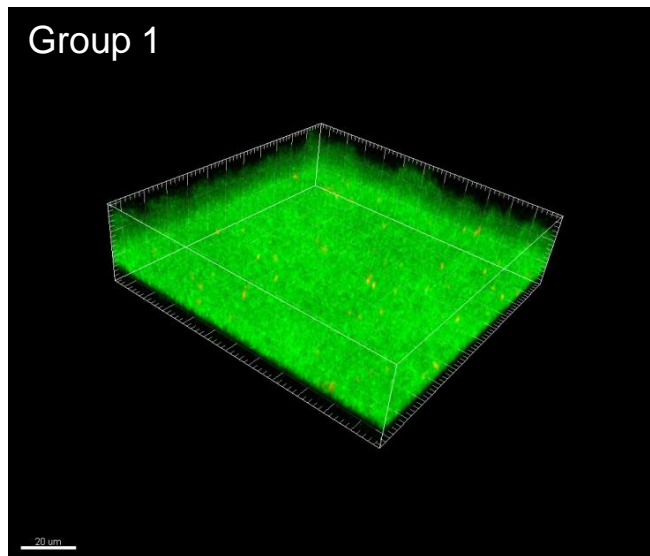
Biomass distribution in mixed biofilms



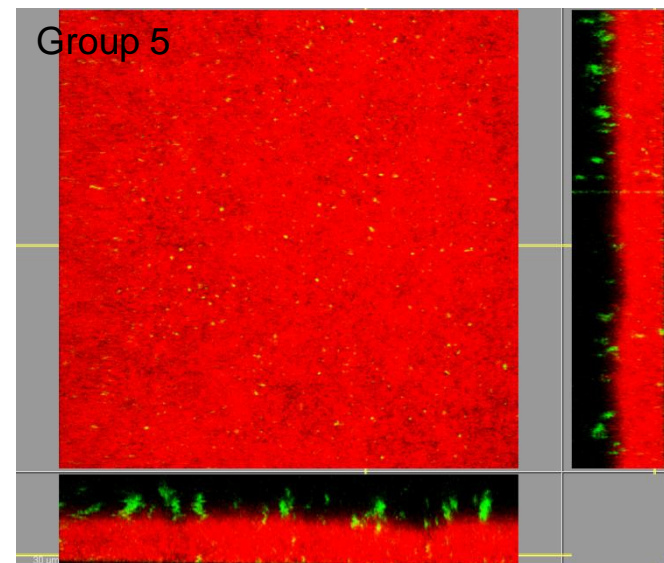
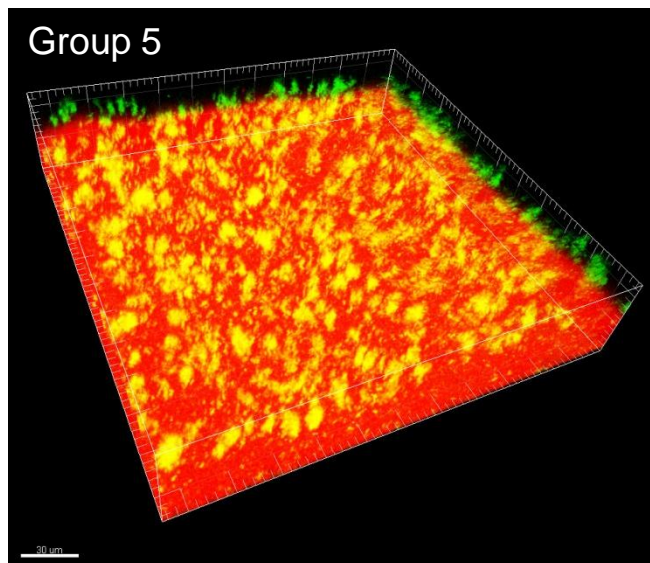
Colistin tolerance in *P. aeruginosa* biofilms

- Strain: wild type *P. aeruginosa* (*gfp*)
 - One flow channel is exposed to colistin (25 µg/ml, over night)
 - One flow channel is used as a control

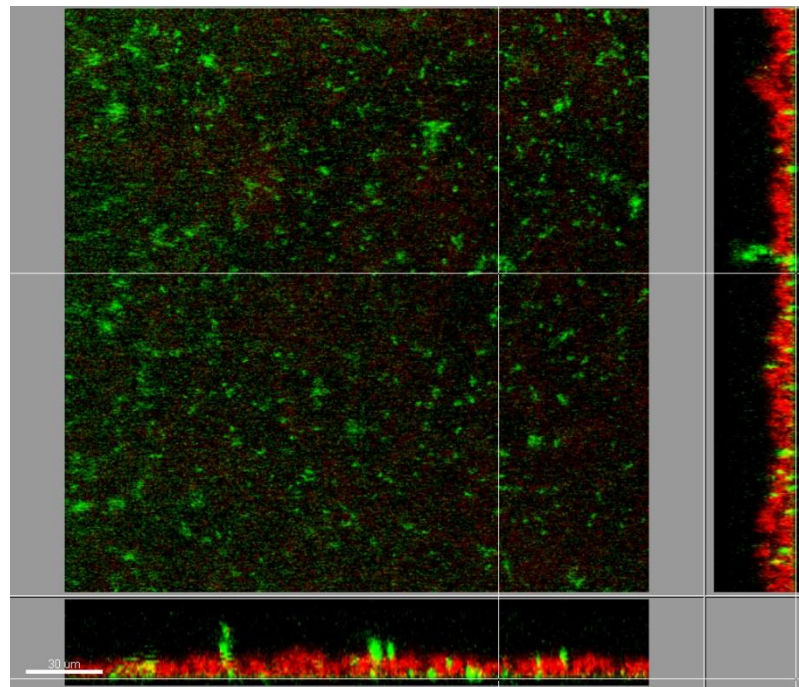
Untreated



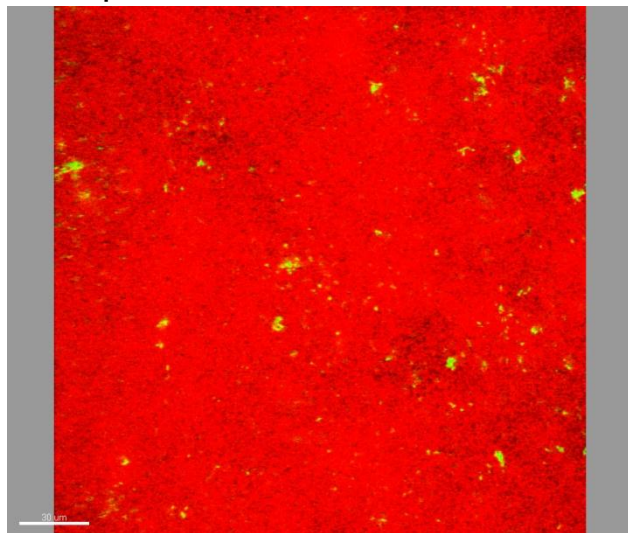
Treated



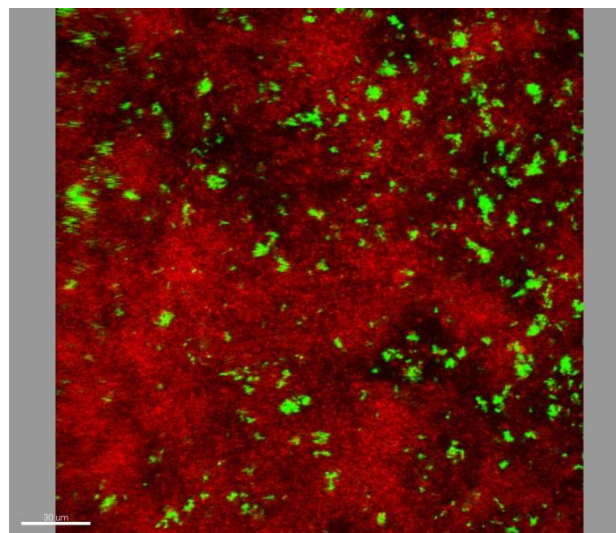
Group 4



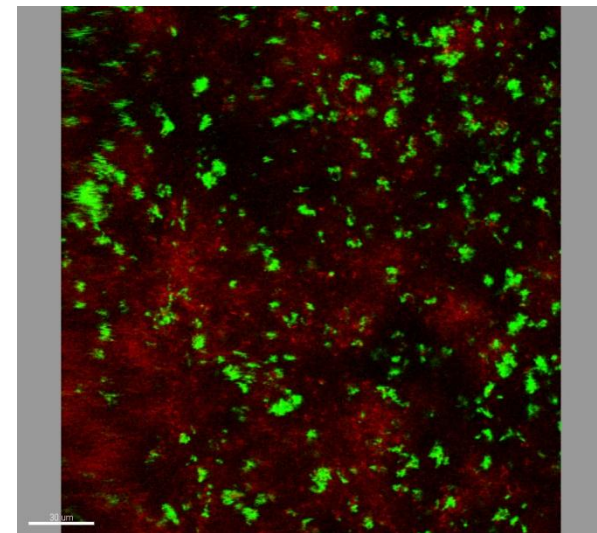
Group 2



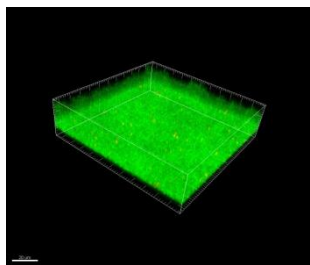
Slice 3



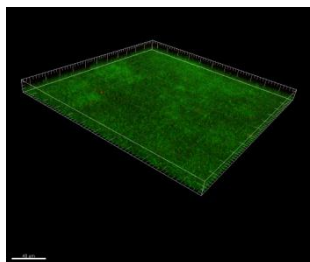
Slice 9



Slice 12

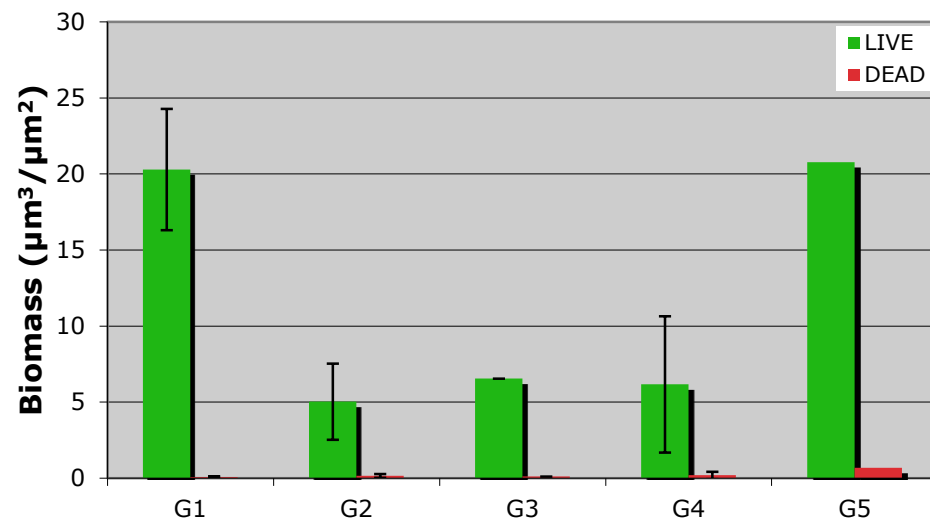


Group 1

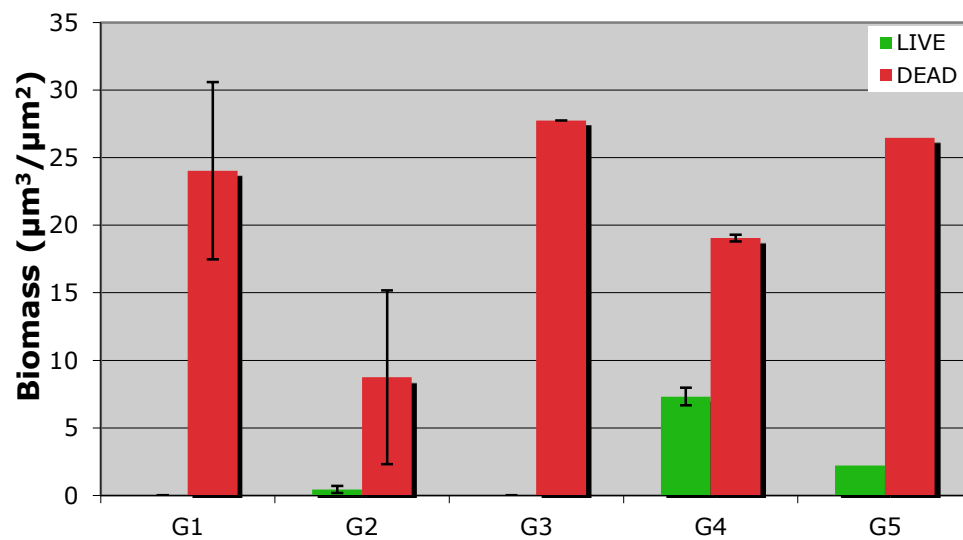


Group 2

Untreated biofilm



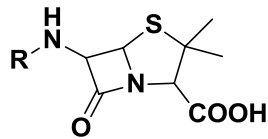
Colistin treated biofilm



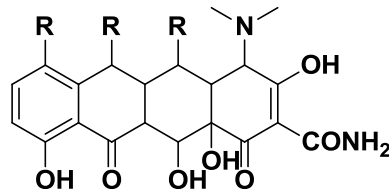
Free Exercise

Group 1

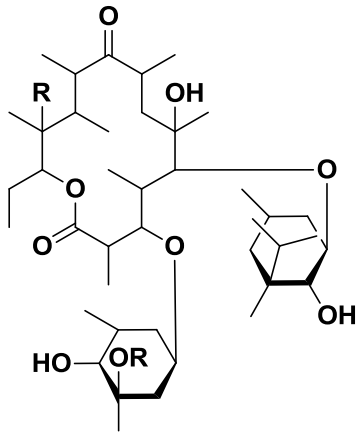
The peptide solution?



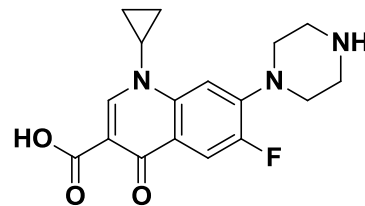
Penicillin (β -lactam)



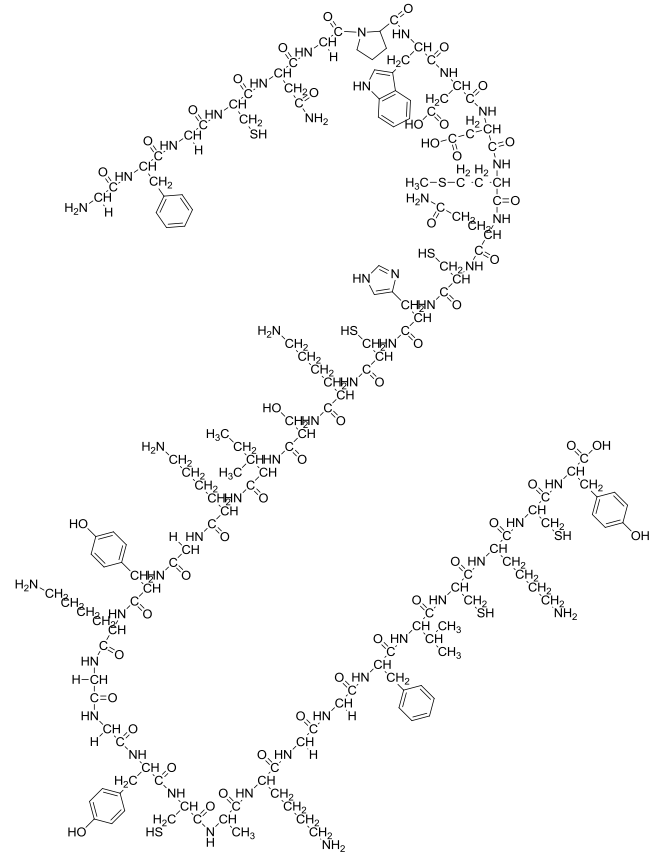
Tetracycline



Erythromycin (macrolide)



Ciprofloxacin (quinolone)



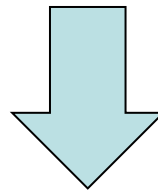
Antimicrobial peptide

Compound 62

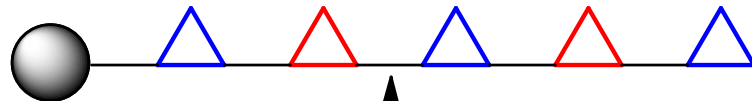
α -amino acid / β -peptoid hybrid

Methodology

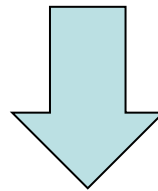
Building block (bb): $\text{P}_N\text{--}\overset{\text{H}}{\text{N}}\text{--aa--OH}$



Solid-phase synthesis:



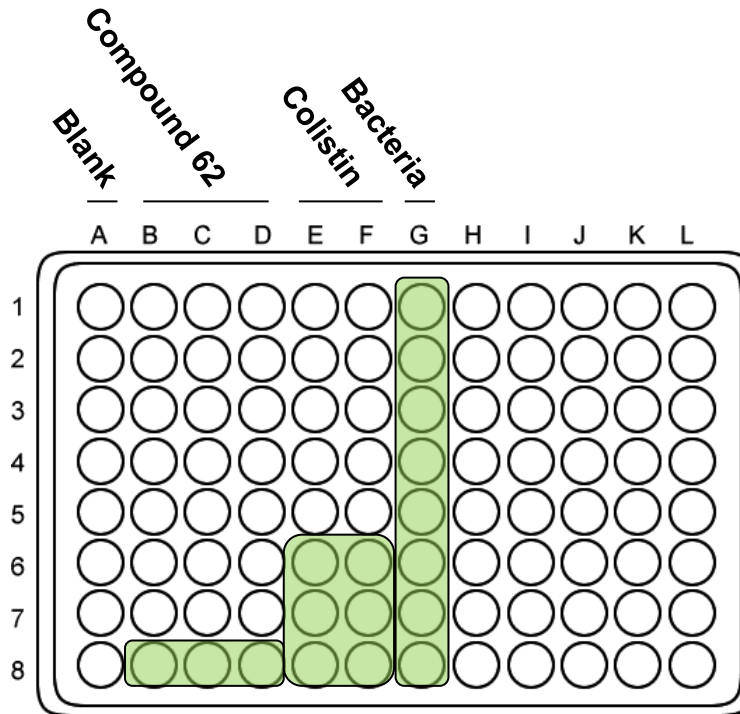
assembly of bb using coupling reagent



Biological investigation

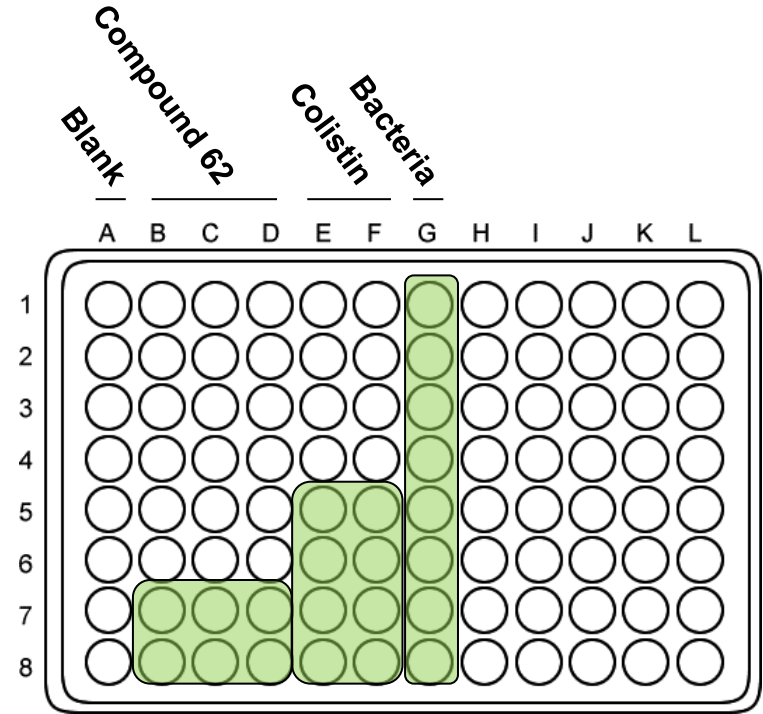
MIC investigation

Concentration gradient



Polypropylene plate

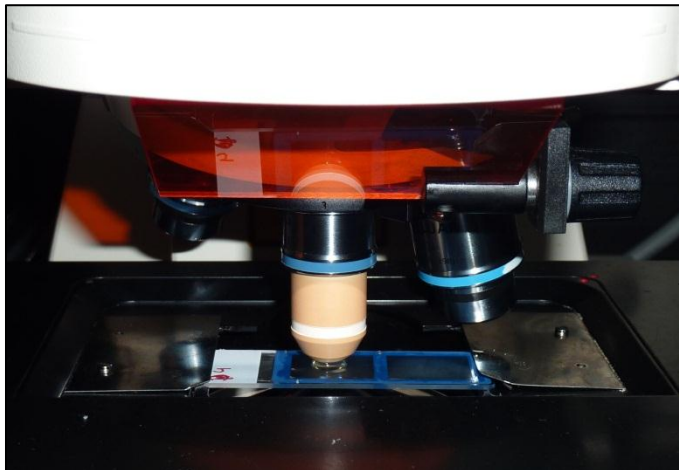
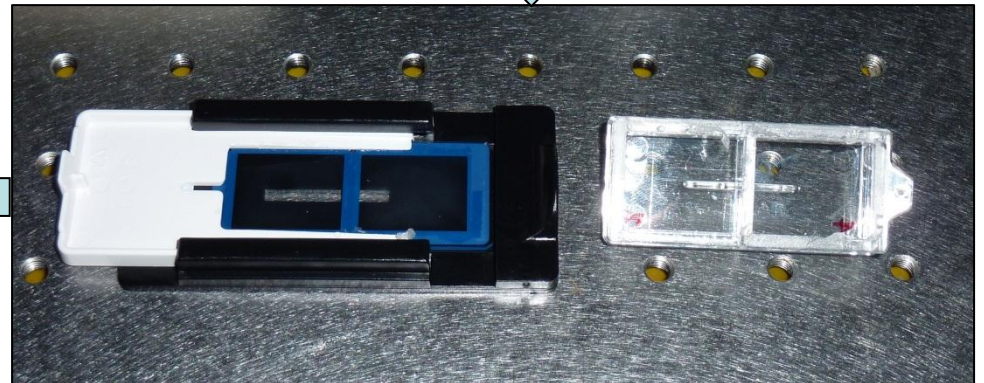
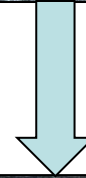
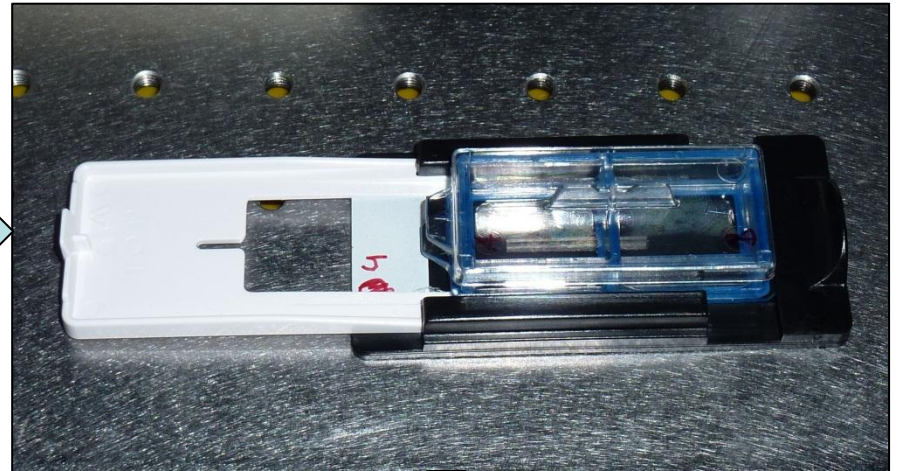
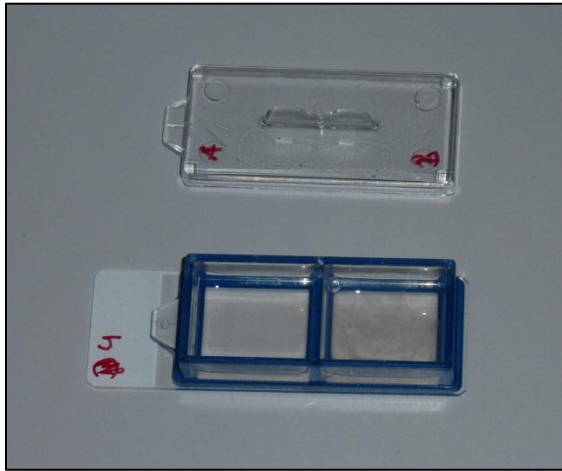
	MIC
Compound 62	1 μ M
Colistin	0.5 μ g/mL



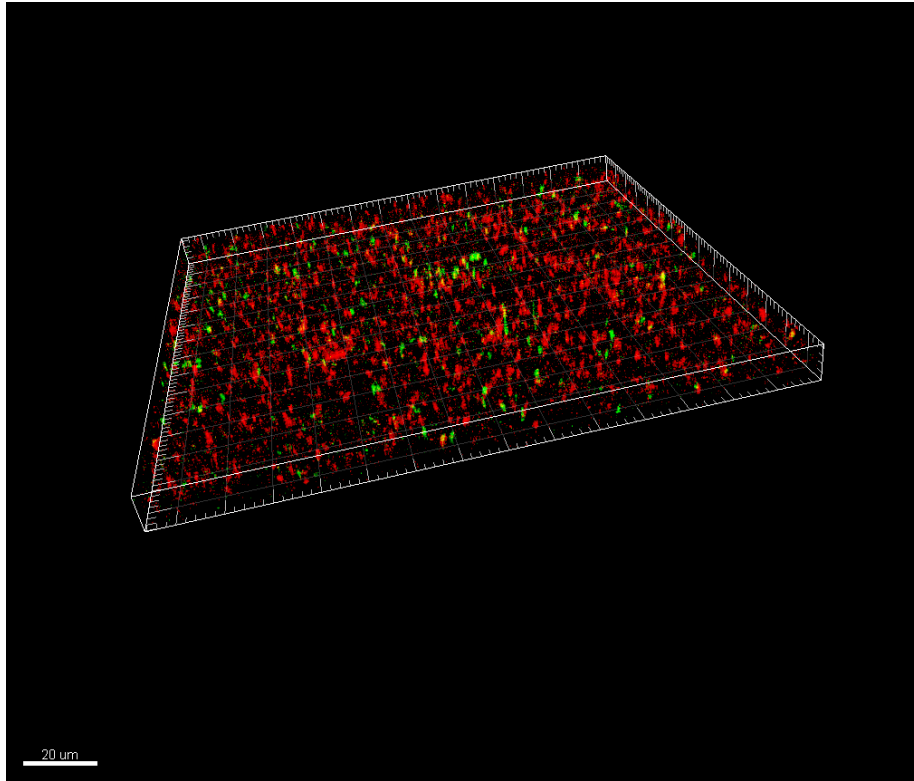
Regular plate

	MIC
Compound 62	2 μ M
Colistin	1 μ g/mL

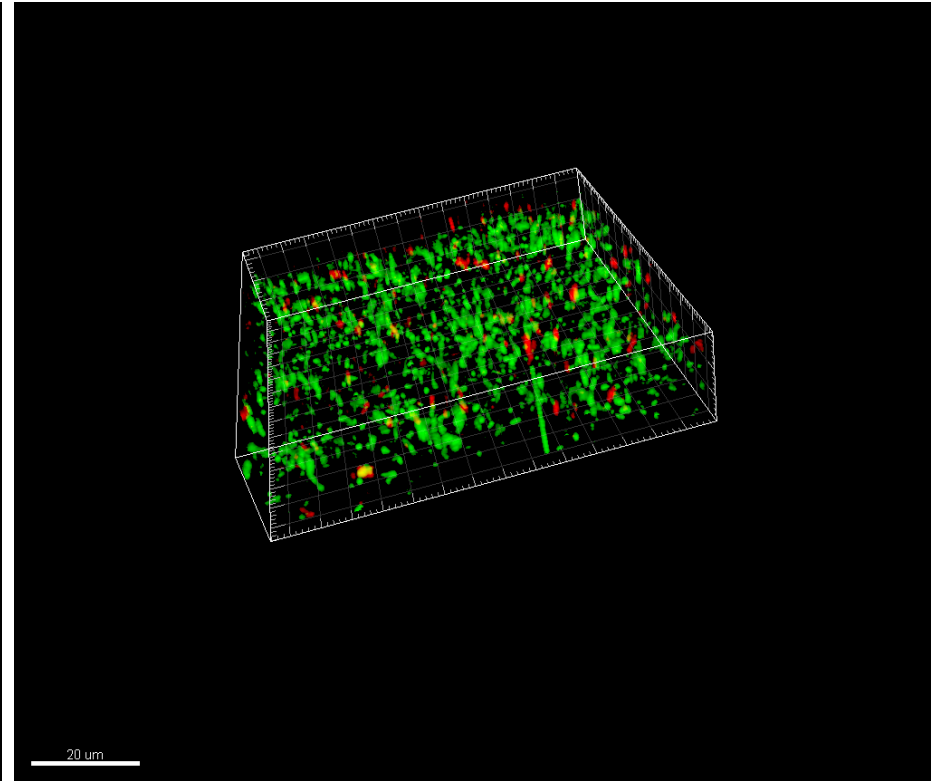
Static chamber system



Results



Treated



Untreated

Conclusions

- The static chamber system is a convenient screening technique.
- Compound 62 is active against biofilm.